Studies on Cadmium-Induced Inhibition of Hepatic Microsomal Drug Biotransformation in the Rat

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Cadmium is a potent inhibitor of hepatic microsomal drug biotransformation in the rat. Male rats receiving a single intraperitoneal dose of cadmium exhibit significant decreases in hepatic microsomal metabolism of a variety of substrates. The threshold cadmium dose is 0.84 mg Cd/kg, and the effect lasts at least 28 days. Mechanistically, the inhibitory effect results from decreased cytochrome P-450 content since cadmium does not alter NADPH cytochrome c reductase activity. This effect is also observed following acute oral administration of cadmium in doses greater than 80 mg Cd/kg but is not observed following chronic administration of the metal via drinking water in concentrations of 5–200 ppm for periods ranging from 2 to 50 weeks. A tolerance to the inhibitory cadmium effect is observed if male rats are pretreated with subthreshold doses of the metal prior to the challenge cadmium dose. The degree of tolerance can be overcome by increasing the challenge dose of cadmium. Characterization of the tolerance phenomenon in terms of onset, duration, and intensity reveals a good correlation with the kinetics of metallothionein production, suggesting that the underlying basis for the tolerance phenomenon is likely the induction of metallothionein. A sex-related difference in the inhibitory effect of cadmium was observed. Cadmium did not inhibit the metabolism of hexobarbital or ethylmorphine in female rats but did inhibit that of aniline or zoxazolamine. Cadmium did not lower cytochrome P-450 content in female rats.

Introduction

Within the last decade, several investigators have found that many of the heavy metals, considered to be environmental pollutants, possess the ability to inhibit the hepatic microsomal enzyme system responsible for the biotransformation of hormones, drugs, and xenobiotics. This may be of considerable importance since alterations in this enzyme system could alter the toxic potential of a wide variety of other substances with which the biological system comes into contact.

Our laboratory has been engaged in studying the toxicity of cadmium with particular interest in the effect of this metal on the mixed-function oxidase system (*1*) located in hepatic microsomes since this heavy metal accumulates in rather high concentrations in the liver and induces hepatotoxicity. This communication describes various studies concerned with the inhibitory effects of cadmium on the hepatic microsomal drug metabolizing system in rats.

Materials and Methods

Male Sprague-Dawley derived rats (Laboratory Supply Co., Indianapolis) were used throughout these studies. The animals were housed in community cages for 10-14 days in air-conditioned rooms with controlled light-dark cycles (12:12) and free access to food and water.

Cadmium acetate (Fischer Scientific Co., Fair Lawn, N. J.) was administered in varying doses by parenteral or oral routes or in the drinking water. Controls received sodium acetate (NaAc) in such a dose or concentration as to receive acetate ion equimolar to the acetate concentration in the cadmium solutions.

Hexobarbital (100 mg/kg, IP) hypnosis was measured as the time elapsing between the loss and re-

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gaining of the righting reflex. Hepatic drug metabolism of various substrates was measured using the $105,000g_{max}$ microsomal pellet as previously described (2, 3). Substrates used were: hexobarbital, determined by the method of Brodie et al. (4); ethylmorphine, assessed by determining the amount of formaldehyde formed using a modification of the method of Nash (5) as described by Anders and Mannering (6); and aniline, determined by the method of Imai et al. (7). Unless stated otherwise enzyme activity was expressed as: hexobarbital, nmoles hexobarbital metabolized/mg microsomal protein/20 min; ethylmorphine, nmoles formaldehyde formed/mg microsomal protein/15 min; and aniline nmole p-aminophenol formed/mg microsomal protein/20 min. Cytochrome P-450 content in microsomes was estimated by the method of Omura and Sato (8). Hepatic metallothionein concentrations in the $105,000g_{max}$ supernatant were measured by the method of Probst et al. (9), which measures metallothionein and total cadmium binding capacity of the metallothionein.

The data were analyzed statistically by use of analysis of variance followed by application of Newman-Keuls test where appropriate or by use of Dunnett's multiple comparisons test (10).

Results and Discussion

Inhibition of Hepatic Microsomal Drug Metabolism by Cadmium

Following the parenteral administration of cadmium in doses ranging from 0.84 to 2.30 mg /Cd/kg, male rats exhibit significantly prolonged responses to drugs (1–3, 11) consequent to a significant inhibition of hepatic microsomal metabolism of a wide variety of substrates (1–3, 11–14). Dose-response studies indicate that the threshold dose for this effect is 0.84 mg Cd/kg, by the intraperitoneal route (11). Interestingly, doses of cadmium greater than this dose do not produce any greater inhibition of this enzyme system. As shown in Figure 1, the magnitude of inhibition for each of three substrates, hexobarbital, ethylmorphine, or aniline, did not differ when comparing cadmium doses of 0.84 and
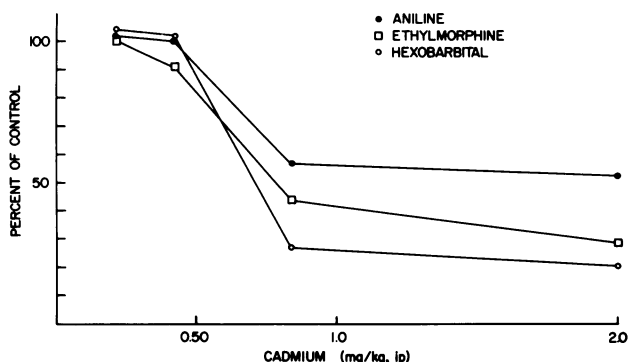

FIGURE 1. Effect of varying doses of intraperitoneally administered drug metabolism in the rat. Metabolism assays were conducted 72 hr after cadmium treatment. Asterisks (*) denote values significantly different from control ($p < 0.05$).
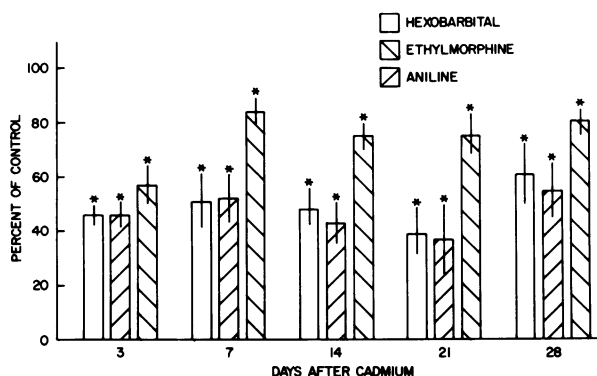

FIGURE 2. Duration of inhibition of hepatic microsomal drug metabolism following cadmium (0.84 mg Cd/kg, IP) administration. Asterisks (*) denote values significantly different from control ($p < 0.05$).

Table 1. Effect of cadmium on enzyme activity and cytochrome P-450 content in male rat hepatic microsomes.

	Microsomal enzyme activity								
	Per (mg microsomal protein)/20 min			Per (nmole cytochrome P-450)/20 min			Cytochrome P-450, nmole/mg microsomal protein		
Measurement	NaAc	CdAc	% Change	NaAc	CdAc	% Change	NaAc	CdAc	% Change
Substrate									
Hexobarbital	266 ± 15	55 ± 6[b]	−79	209 ± 12	72 ± 8[b]	−65			
Ethylmorphine	347 ± 17	100 ± 4[b]	−71	272 ± 13	131 ± 5[b]	−52			
Aniline	31 ± 1	17 ± 1[b]	−47	25 ± 1	22 ± 1	−11	1.25 ± 0.04	0.76 ± 0.02[b]	−39

[a] Male rats received cadmium (2.0 mg Cd/kg, IP) and 72 hr later measurement of hepatic drug metabolism, spectral binding, or cytochrome P-450 content was conducted. From Means et al. (15).
[b] Significantly different from control ($p < 0.05$).

2.0 mg Cd/kg (IP). However, differences were observed in the amount of inhibition when the substrates were compared. Similar results were found when hexobarbital hypnosis was used in that doses of cadmium between 0.84 and 2.5 mg Cd/kg, (IP) produced about the same degree of potentiation of duration of hypnosis (1, 11).

The duration of the inhibitory effect of cadmium has been reported to vary. Most experiments were of short duration of less than 7 days. Hadley et al. (2) reported that cadmium potentiated duration of hexobarbital hypnosis for 10 days, but values had returned to control levels by 17 days. However, we have found that a single acute dose of 0.84 mg Cd/kg given by the intraperitoneal route inhibited microsomal enzyme activity using three different substrates for at least 28 days, the longest time interval examined (Fig. 2). As indicated, the magnitude of inhibition exhibited by cadmium tends to vary with the substrate used. In this regard, typical results are presented in Table 1 for hexobarbital, ethylmorphine, and aniline as representative examples of type I (HS, EM) or type II (AN) substrates, respectively. The magnitude of inhibition elicited by cadmium treatment was 1.5 to 1.7 times greater for type I substrates than for type II. Cadmium also depressed the level of microsomal cytochrome P-450 content (Table 1) but exerted no significant effect on NADPH cytochrome c reductase activity (226 ± 9 vs. 187 ± 36 nmole cytochrome c reduced/mg protein/min).

From these data, the inhibitory effect of cadmium on hepatic microsomal drug metabolism most likely results from decreasing the availability of the microsomal hemoprotein, cytochrome P-450. Furthermore, if the enzyme activities are expressed on the basis of cytochrome P-450 content, microsomal aniline hydroxylase activity in cadmium-treated rats was not significantly (11%) altered, while cadmium-induced inhibition of the rate of hexobarbital metabolism and ethylmorphine was still significant (65% and 52%, respectively) though of lesser magnitude. Significant decreases (30–40%) in microsomal cytochrome P-450 content have been reported by others (1, 2, 13–15). Additional studies by Krasny and Holbrook (13) indicate that cadmium induces microsomal heme oxygenase (350%), the first enzyme responsible for heme degradation. Correlated with the induction of this enzyme, there is an increase in the degradation of cytochrome P-450 as assessed by measuring the decline of ^{3}H-δ-aminolevulinic acid from microsomal CO-binding particles (13, 16).

Since the previous studies were concerned with the parenteral administration of cadmium, we examined the effect of acute oral cadmium adminis-

tration on drug response and metabolism. The data shown in Figure 3 indicate that cadmium also effectively potentiates duration of hexobarbital hypnosis in a dose-response relationship with the threshold cadmium dose being 80 mg Cd/kg, and the magnitude of potentiation increasing with increasing cadmium doses (Fig. 3). Similar results were found when cadmium inhibition of microsomal metabolism of hexobarbital and aniline was examined (17).

Thus, following the acute administration of cadmium by either parenteral or oral routes, there is a rather clear threshold dose required to alter hepatic drug metabolism. Interestingly, the oral/intraperitoneal dose ratio is about 100/1, a value which correlates well with the observation that only 1–2% of an oral cadmium dose is absorbed (18). Our findings with oral cadmium administration differ from the data of Kotsonis and Klaassen (19), who administered cadmium chloride orally in doses ranging from 0 to 150 mg Cd/kg but found no alterations in microsomal cytochrome P-450 content or aniline hydroxylase activity at 2 or 4 days after cadmium. These investigators did find a slight decrease in hexobarbital oxidase activity at 2 days but not 14 days after cadmium. At present, we have no explanation for these differences in observations.

The effect of chronic cadmium administration on hepatic drug metabolism was investigated by giving cadmium in the drinking water. In the first study, cadmium was administered in concentrations of 100–200 ppm for periods of 2-12 weeks. From the data in Table 2, it is readily apparent that there were no alterations in drug response or hepatic drug metabolism in spite of the observation that the total cadmium intake was much greater than the acute oral dose required for altering enzyme activity. In another study of similar design, rats received cadmium in the drinking water at concentrations of 5 or 20 ppm for 50 weeks. Again, no changes in drug response or metabolism were found (17). Similar results have been reported by Becking (20), who administered cadmium (to rats) in drinking water at concentrations of 2–200 ppm for 60–180 days and found no change in hepatic drug metabolizing activity or cytochrome P-450 content.

Although no studies have yet been directed to resolve these differences between the acute and chronic effects of orally administered cadmium, they are likely related to cadmium levels reaching the liver. As indicated, about 1–2% of an oral cadmium dose is absorbed (18). In addition, the ability of cadmium to induce the cadmium-binding protein, metallothionein, in various tissues offers a further complicating factor. Thus, metallothionein induced in the intestinal mucosa (21) may decrease the ab-

sorption of the metal and metallothionein in the liver (9) may bind cadmium in an inert form, thus, preventing toxicity.

Tolerance to the Cadmium-Induced Inhibitory Effects

Pretreatment of animals with subtoxic doses of cadmium has been shown to prevent various toxic syndromes attributed to higher doses of cadmium (22–26). In our laboratory we have found that if subthreshold doses of cadmium (0.21 or 0.42 mg Cd/kg, IP) are administered prior to the threshold cadmium (0.84 mg Cd/kg, IP) dose, the cadmium-induced inhibition in drug metabolism is no longer observed (1, 26). Correlated with this tolerance phenomenon were increased hepatic concentrations of metallothionein offering support for the protective role of this cadmium-binding protein (27). A series of experiments were conducted to charac-

terize this tolerance phenomenon with respect to onset, duration, and level of intensity. The minimum time period for a subthreshold dose of cadmium (0.21 mg/kg, IP) to produce a tolerance to the threshold cadmium dose was 14 hr, and the phenomenon lasted for at least 336 hr, the longest time interval measured when either duration of hexobarbital hypnosis or hepatic drug metabolism was used (1, 27). The peak intensity of the tolerance occurred at 72 hr. The minimal tolerance producing dose of cadmium was 0.1 mg Cd/kg for the challenge (0.84 mg Cd/kg) cadmium dose (27). We also found that the intensity of tolerance was finite. In this experiment, the tolerant state was induced by administering cadmium (0.21 mg/kg) and 72 hr later the animals were challenged with cadmium in doses ranging from 0.84 to 3.2 mg Cd/kg. From Figure 4, it can be seen that tolerance was no longer apparent in animals receiving cadmium in doses greater than 2.0 mg Cd/kg, when considering the average values for each cadmium dose. Interestingly, however, when the individual data points were plotted, it would appear that there are at least two different populations of animals per challenge dose, i.e., a group of rats that were nontolerant and a group of tolerant rats. The nature of this individual variability is not known.

The role of metallothionein in this tolerance phenomenon was assessed by comparing the kinetics of synthesis of the protein with the temporal development of the tolerance phenomenon. Rats received cadmium (0.21 mg Cd/kg) and were sacrificed at varying intervals thereafter. Total metallothionein concentrations and cadmium-binding capacity of the protein increased rapidly and were maximal from 8 to 67 hr but remained above control values from 67 to 240 hr, declining thereafter (27). Thus, these data correlate well with the temporal development and duration of the tolerance phenomenon reported in previous experiments.
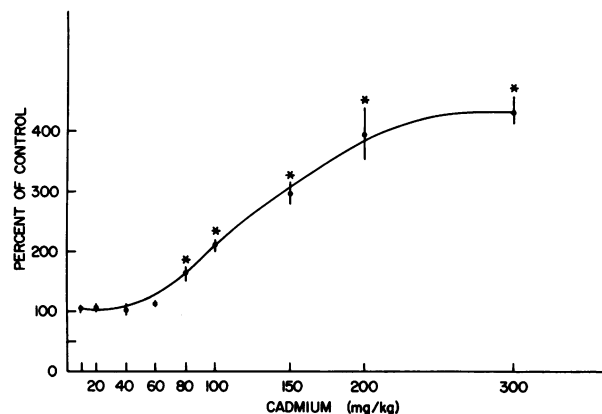

FIGURE 3. Effect of varying doses of orally administered cadmium on duration of hexobarbital hypnosis. Experiments were conducted 72 hr after cadmium treatment. Asterisks (*) denote values significantly different from control ($p < 0.05$).

Table 2. Effect of chronic oral cadmium administration on duration of hexobarbital hypnosis and hepatic microsomal drug metabolism in male rats.[a]

Treatment Cd, ppm	Duration of treatment, weeks	Total Cd consumed, mg	Duration of hexobarbital hypnosis, min ± S. E.	Hepatic hexobarbital, μmole metabolism mg protein-hr
0	12	0	19.2 ± 1.1 (6)	1.24 ± 0.12 (6)
100	2	43.1 ± 2.3	17.9 ± 1.8 (8)	1.43 ± 0.11 (8)
100	4	89.6 ± 2.5	16.5 ± 0.9 (7)	1.13 ± 0.13 (6)
100	12	233.5 ± 19.8	16.4 ± 1.5 (6)	1.04 ± 0.08 (6)
200	2	61.2 ± 3.3	21.7 ± 3.1 (7)	1.10 ± 0.10 (6)
200	4	154.9 ± 77.6	18.2 ± 0.8 (7)	1.12 ± 0.07 (6)

[a] Adult, male rats were housed individually, and cadmium was administered in the drinking water in the concentrations and for the time intervals as designated controls received sodium acetate. Hypnosis was induced by hexobarbital (100 mg/kg, IP). Hexobarbital metabolism was measured in hepatic microsomes as described. Adapted from Schnell et al. (18).

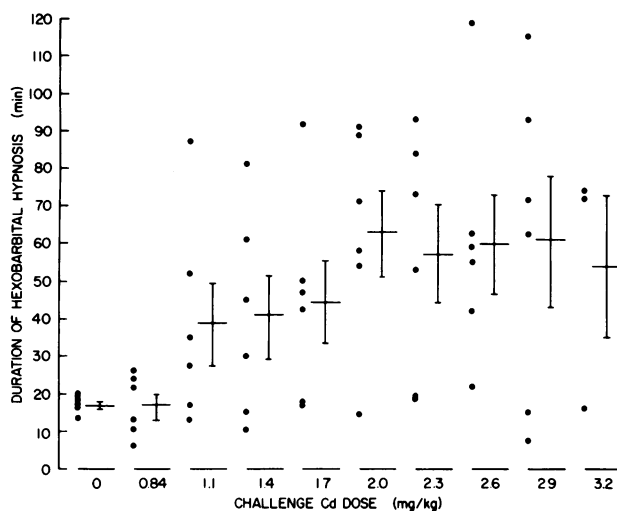**Environmental Health Perspectives**

FIGURE 4. Alteration of cadmium-induced tolerance phenomenon. Male rats received a tolerance-producing cadmium dose (0.21 mg Cd/kg, IP) and 3 days later were challenged with increasing cadmium doses and duration of hexobarbital hypnosis was measured 72 hr later. The data are plotted as individual points (filled circles) and as average values (horizontal bar) ± S.E.M. Asterisks (*) denote values significantly different from control ($p < 0.05$).

Sex-Related Differences in Cadmium Inhibition of Hepatic Drug Metabolism

In previous studies we have reported that cadmium-induced inhibition of hepatic drug metabolism in the rat is sex-related with cadmium exerting no inhibitory effects in the female (1, 3, 28). Dose-response studies revealed that lethal doses of cadmium were ineffective in potentiating the duration of hexobarbital hypnosis in female rats (3). These sex-related differences in the cadmium effect are apparently substrate-dependent, as shown in Figure 5. When the effect of cadmium on hepatic metabolism of hexobarbital, ethylmorphine, aniline, or zoxazolamine was assessed in male and female rats, a significant cadmium inhibition in the metabolism of all four substrates was observed in the male rats. In female rats, no inhibition was observed in the

metabolism of hexobarbital or ethylmorphine; however, a significant inhibition was elicited by cadmium in the metabolism of aniline and zoxazolamine but of lesser magnitude than the inhibition observed in the males. Hexobarbital, ethylmorphine, and zoxazolamine are type I substrates and the metabolism of hexobarbital and ethylmorphine show sex-dependent characteristics, while that of zoxazolamine is sex-independent. Aniline is characterized as a type II, sex-independent substrate. In additional studies, there was no effect of cadmium on cytochrome P-450 content in female rats (29). Since sex-dependent hepatic drug metabolism is apparently controlled by the influence of the male sex hormone, testosterone, on enzyme activity (30), it was of interest to determine if these sex-related differences to cadmium observed in the rat were androgen sensitive and mediated via cadmium-induced testicular necrosis (22). In this experiment the effect of cadmium on hepatic drug metabolism and drug responses was examined in castrated male rats. The data presented in Table 3 indicate that cadmium administered to castrated rats potentiated duration of hexobarbital hypnosis only 54% compared to 235% in sham-operated males, and inhibited hepatic metabolism of hexobarbital only 39% in castrated rats compared to 75% in controls. With aniline as the substrate, no inhibition in hepatic drug metabolizing enzyme activity was observed in the castrated rats treated with cadmium. Thus, these results indicate that castration affords partial protection against cadmium-induced inhibition of hepatic drug metabolism. In addition, experiments, we have found that cadmium exerts no inhibitory effects on hepatic drug metabolism in immature male or female rats (29).

Conclusion

Cadmium is a potent inhibitor of hepatic microsomal drug metabolism in the male rat. The inhibition results from a decrease in the microsomal content of cytochrome P-450. Clear threshold relation-

Table 3. Effect of cadmium treatment on drug response and hepatic microsomal drug metabolism in castrated male rats.[a]

| | Duration of hexobarbital hypnosis, min ± S.E. | | Hepatic microsomal drug metabolism | | | |
| | | | Hexobarbital | | Aniline | |
Sex	NaAc	CdAc	NaAc	CdAc	NaAc	CdAc
Sham	20.8 ± 1.5	69.7 ± 6.3[b]	1.06 ± 0.06	0.27 ± 0.07[b]	60.4 ± 1.4	49.1 ± 4.5[b]
Castrated	31.6 ± 3.5	48.8 ± 6.1[b]	0.82 ± 0.05	0.50 ± 0.09[b]	60.6 ± 2.9	54.7 ± 2.0

[a] Sham-operated and castrated male rats received cadmium (2 mg/kg, IP) and 72 hr later duration of hexobarbital hypnosis or microsomal metabolism of hexobarbital or aniline was measured.
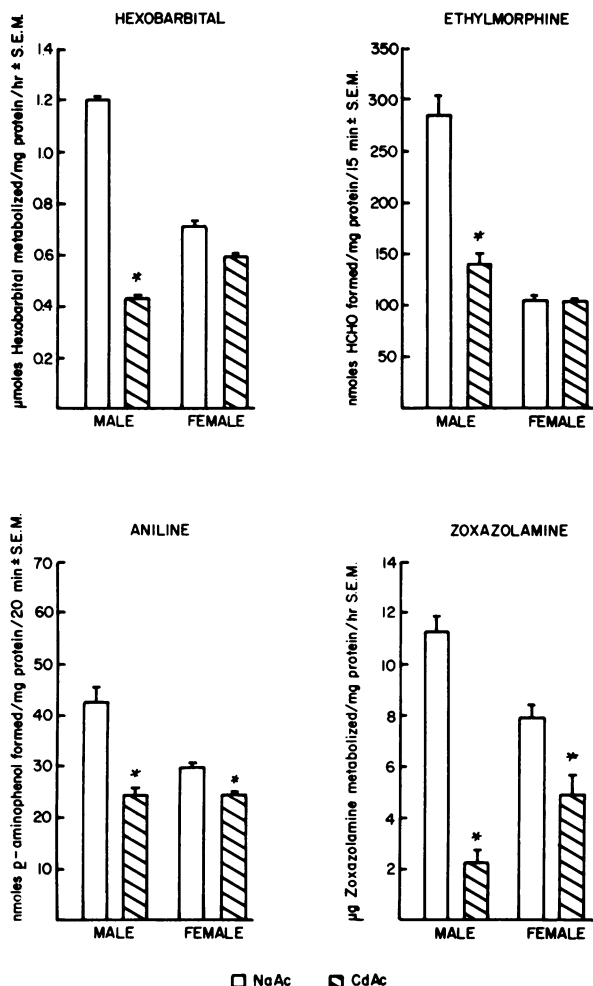[b] Significantly different from respective control ($p < 0.05$).

FIGURE 5. Effect of cadmium on hepatic microsomal drug metabolism in male and female rats. Male or female rats received cadmium (2.0 mg Cd/kg, IP), and hepatic microsomal metabolism was measured 72 hr later. Asterisks (*) denote values significantly different from controls ($p < 0.05$).

ships have been defined for the inhibitory effects to be manifested after acute cadmium administration despite route of administration of the metal. Administration of cadmium in doses ineffective to inhibit drug metabolism produces a tolerance to the subsequent administration of an effective challenge cadmium dose. The underlying basis of this tolerance is probably the induction of metallothionein, a cadmium-binding protein. Chronic administration of the metal in drinking water does not alter hepatic drug metabolism, probably as a result of metallothionein induction. The inhibitory effect of cadmium toward the hepatic microsomal drug metabolizing enzyme system in the rat shows sex-related characteristics. Although the metabolism of all drug substrates is inhibited in male rats, such

inhibition is exhibited in female rats only for those substrates which are sex-independent in terms of metabolic rate.

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